

(1) 申請者	日本特許庁 (JP)	(2) 公開特許公報 (A)	(3) 公開日	平成14年5月9日(木)
(5) 国名	日本	(6) 領別記号	P I	フジト
A 61 K	39/00	A 61 P	29/00	101
A 61 P	29/00		35/00	4 C
	35/00		43/00	
	43/00	A 61 K	37/02	

特許2000-316484(P2000-316484)	(71)出願人 正烟 伊成 東京都文京区向丘1-20-6-503
平成12年10月17日(2000.10.17)	(72)発明者 正烟 伊成 東京都文京区向丘1-20-6-503
	(74)代理人 アリミ士 孖寶 三幸 (4名) Pタム(参考) 40084 A02 AG3 BA44 DA184 MA02 NM5 ZB152 ZZ282
(21)出願番号 平成22年10月10日	ZC12 ZCT52

卷之三

37) 【要約】 TNF- $\alpha$ 及びIL-4を有効成分とする抗アレルギー薬剤。本明アボーシス株式会社が開発され、副作用の少ない制癌剤、慢性的な炎症が原因のアマチ治愈剤、肝炎、肝硬変等の肝疾患治療剤として使用できる。

このようにして、アボリーション運動が、NFT-a及びL-4に対しては、それがNFT-a及びL-4としての活性を示す。天板部が、け顔部子供様により產生された角角体に向むかひで包含される。

[0011] 天然型のTNF- $\alpha$ は、例えは、セントライウイルス (Sentai Virus) 初値にトボソウ特異的 RNA LL-1などの既存の細胞株の活性上昇より、アフニティーカロマトグラフィーや I PLCなどの既知方法によれば得るなどにより得ることが可能。遺伝子から組み立てて得るなどにより得ることが可能。

(00107) 細胞の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えば乳癌、白癌、癌ナトリウム、アドレナリノン、テンブン、燃焼カルシウム、カナリーン、精蟲セルロース、ケイ酸等の直形剤、水、エタノール、プロパンノール、酢酸アップ、ブドウ糖液、デンブン液、ゼラチン液等、点詰剤等が挙げられる。

(00108) 細剤の形態に成形するに際しては、相体として前記と同様にして供することが可能である。

(00109) 本発明のアボトーシス精制剂は、ヒトT細胞クローニングや未梢T細胞感覚性細胞をマイクロソーナー等で非標的感覚性細胞から導入に用いて得られ、組成は、骨髓異形成症保群、周囲性血小板減少症、再生不良性貧血、突発性血小板減少症等を含む、各種の疾患、アルツハイマー型、A型、B型、F型等の各種の肝炎、アルツハイマー病、アルツハイマー型老年痴呆症、心筋炎、ARDS (肺)、呼吸困難症候群等が例示できる。更に該剤は必要に応じ通常の薬液等が例示できる。例えば輸衣鉢、ゼラチン被包錠、脚部被包錠、フルムコロニング錠あるいは二重錠、多層錠等が例示できる。

(00110) 丸剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えばブドウ糖、カカオオイル、硬化植物油、カオチアゴム、アラビアゴム末、トラガントキモ、ゼラチン、エタノール等の結合剤、ミナミカンタン等の増強剤等が例示できる。

(00111) 丸剤の形態に成形するに際しては、相体として後半段のものを広く使用でき、例えばポリエチレンゴルム、カカオ、高級アルコール、高級アルコールのエタノール、乳剤等、セラチン、半合成セラライド等を挙げることができる。

(00112) 丸剤の形態として調製される本発明のアボトーシス精制剂は、後記実験例に示すようにTNF-a又はIL-4を単独で用いた場合に比べ、アボトーシス精制剂が著しく増強されるという相乗作用を発揮する場合にて、TNF-a又はIL-4を単独で投与する場合に比べて両者の投与量を大幅に減少させることができ、副作用の質感が可能となる。

(00113) 本発明のアボトーシス精制剂の成人に対する一日当たりの投与量は、通常一日に5.0mg/kg～5.0mg/body程度であり、IL-4については、一日当たり5.0μg/body程度とするのが宜しい。

(00114) 本発明のアボトーシス精制剂は、その適用目的に応じ、医療製剤としてこの分野で用いられている各種の投与形態で使用される。断然使用される製剤は、断端剤、栓塞剤、封入剤、付添剤、崩壊剤、表面活性剤、被覆剤等の殺細胞剤は無毒性毒理相としてして調製される。断端は、治療目的に応じて各種の形態が選択でき、この代表的なものとして鉈剤、丸剤、栓剤、液剤、乳剤、カプセル等が挙げられる。

(00115) 鉈剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えば乳癌、白癌、癌ナトリウム、アドレナリノン、テンブン、燃焼カルシウム、カナリーン、精蟲セルロース、ケイ酸等の直形剤、水、エタノール、プロパンノール、酢酸アップ、ブドウ糖液、デンブン液、ゼラチン液等、点詰剤等が挙げられる。

(00116) 鉈剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えばAIDS、AIDS治療剤、ATL (成人T細胞白血病-Adult T-cell leukaemia)、毛髪細胞白血病 (Hairy cell leukemia)、腫瘍細胞 (HAT/TSP)、呼吸器難病 (HAB/HABA)、関節症 (HAP)、ブドウ糖炎 (HAD)等のHTLV-1関連疾患、自己免疫疾患、例えばSLE (全身性エリテマトーデス)、AIDS、ATL等の膠原病、慢性大腸炎、シェーグレン症候群、原発性腎計性小板減少症等が挙げられる。

(00117) 鉈剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えば乳癌、白癌、癌ナトリウム、アドレナリノン、テンブン、燃焼カルシウム、カナリーン、精蟲セルロース、ケイ酸等の直形剤、水、エタノール、プロパンノール、酢酸アップ、ブドウ糖液、デンブン液、ゼラチン液等、点詰剤等が挙げられる。

(00118) 丸剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えばブドウ糖、カカオオイル、硬化植物油、カオチアゴム末、アラビアゴム末、セラチン等の結合剤、ミナミカンタン等の増強剤等が例示できる。また、G-CSF (granulocyte-macrophage colony-stimulating factor)、TNF-a及びIL-4は、アボトーシス精制剂 (Propranolol) の後フローサイトメーター (EPICS XL: Coulter) にて染色性細胞 (アボトーシス精制剂) を検出し、結果を表1に示す。尚、培地は、RPMI-1640培地 (Life Technologies社製) を使用して用いた。

(00119) 丸剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えばブドウ糖、カカオオイル、硬化植物油、カオチアゴム末、アラビアゴム末、セラチン等の結合剤、ミナミカンタン等の増強剤等が例示できる。また、G-CSF (granulocyte-macrophage colony-stimulating factor)、TNF-a及びIL-4は、アボトーシス精制剂 (Propranolol) の後フローサイトメーター (EPICS XL: Coulter) にて染色性細胞 (アボトーシス精制剂) を検出し、結果を表2に示す。尚、培地は、RPMI-1640培地 (Life Technologies社製) を使用して用いた。

(00120) 丸剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えばブドウ糖、カカオオイル、硬化植物油、カオチアゴム末、アラビアゴム末、セラチン等の結合剤、ミナミカンタン等の増強剤等が例示できる。また、G-CSF (granulocyte-macrophage colony-stimulating factor)、TNF-a及びIL-4は、アボトーシス精制剂 (Propranolol) の後フローサイトメーター (EPICS XL: Coulter) にて染色性細胞 (アボトーシス精制剂) を検出し、結果を表3に示す。尚、培地は、RPMI-1640培地 (Life Technologies社製) を使用して用いた。

(00121) 丸剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えばブドウ糖、カカオオイル、硬化植物油、カオチアゴム末、アラビアゴム末、セラチン等の結合剤、ミナミカンタン等の増強剤等が例示できる。また、G-CSF (granulocyte-macrophage colony-stimulating factor)、TNF-a及びIL-4は、アボトーシス精制剂 (Propranolol) の後フローサイトメーター (EPICS XL: Coulter) にて染色性細胞 (アボトーシス精制剂) を検出し、結果を表4に示す。尚、培地は、RPMI-1640培地 (Life Technologies社製) を使用して用いた。

(00122) 丸剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えばブドウ糖、カカオオイル、硬化植物油、カオチアゴム末、アラビアゴム末、セラチン等の結合剤、ミナミカンタン等の増強剤等が例示できる。また、G-CSF (granulocyte-macrophage colony-stimulating factor)、TNF-a及びIL-4は、アボトーシス精制剂 (Propranolol) の後フローサイトメーター (EPICS XL: Coulter) にて染色性細胞 (アボトーシス精制剂) を検出し、結果を表5に示す。尚、培地は、RPMI-1640培地 (Life Technologies社製) を使用して用いた。

(00123) 丸剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えばブドウ糖、カカオオイル、硬化植物油、カオチアゴム末、アラビアゴム末、セラチン等の結合剤、ミナミカンタン等の増強剤等が例示できる。また、G-CSF (granulocyte-macrophage colony-stimulating factor)、TNF-a及びIL-4は、アボトーシス精制剂 (Propranolol) の後フローサイトメーター (EPICS XL: Coulter) にて染色性細胞 (アボトーシス精制剂) を検出し、結果を表6に示す。尚、培地は、RPMI-1640培地 (Life Technologies社製) を使用して用いた。

(00124) 丸剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えばブドウ糖、カカオオイル、硬化植物油、カオチアゴム末、アラビアゴム末、セラチン等の結合剤、ミナミカンタン等の増強剤等が例示できる。また、G-CSF (granulocyte-macrophage colony-stimulating factor)、TNF-a及びIL-4は、アボトーシス精制剂 (Propranolol) の後フローサイトメーター (EPICS XL: Coulter) にて染色性細胞 (アボトーシス精制剂) を検出し、結果を表7に示す。尚、培地は、RPMI-1640培地 (Life Technologies社製) を使用して用いた。

(00125) 丸剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えばブドウ糖、カカオオイル、硬化植物油、カオチアゴム末、アラビアゴム末、セラチン等の結合剤、ミナミカンタン等の増強剤等が例示できる。また、G-CSF (granulocyte-macrophage colony-stimulating factor)、TNF-a及びIL-4は、アボトーシス精制剂 (Propranolol) の後フローサイトメーター (EPICS XL: Coulter) にて染色性細胞 (アボトーシス精制剂) を検出し、結果を表8に示す。尚、培地は、RPMI-1640培地 (Life Technologies社製) を使用して用いた。

(00126) 丸剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えばブドウ糖、カカオオイル、硬化植物油、カオチアゴム末、アラビアゴム末、セラチン等の結合剤、ミナミカンタン等の増強剤等が例示できる。また、G-CSF (granulocyte-macrophage colony-stimulating factor)、TNF-a及びIL-4は、アボトーシス精制剂 (Propranolol) の後フローサイトメーター (EPICS XL: Coulter) にて染色性細胞 (アボトーシス精制剂) を検出し、結果を表9に示す。尚、培地は、RPMI-1640培地 (Life Technologies社製) を使用して用いた。

(00127) 丸剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えばブドウ糖、カカオオイル、硬化植物油、カオチアゴム末、アラビアゴム末、セラチン等の結合剤、ミナミカンタン等の増強剤等が例示できる。また、G-CSF (granulocyte-macrophage colony-stimulating factor)、TNF-a及びIL-4は、アボトーシス精制剂 (Propranolol) の後フローサイトメーター (EPICS XL: Coulter) にて染色性細胞 (アボトーシス精制剂) を検出し、結果を表10に示す。尚、培地は、RPMI-1640培地 (Life Technologies社製) を使用して用いた。

(00128) 丸剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えばブドウ糖、カカオオイル、硬化植物油、カオチアゴム末、アラビアゴム末、セラチン等の結合剤、ミナミカンタン等の増強剤等が例示できる。また、G-CSF (granulocyte-macrophage colony-stimulating factor)、TNF-a及びIL-4は、アボトーシス精制剂 (Propranolol) の後フローサイトメーター (EPICS XL: Coulter) にて染色性細胞 (アボトーシス精制剂) を検出し、結果を表11に示す。尚、培地は、RPMI-1640培地 (Life Technologies社製) を使用して用いた。

(00129) 丸剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えばブドウ糖、カカオオイル、硬化植物油、カオチアゴム末、アラビアゴム末、セラチン等の結合剤、ミナミカンタン等の増強剤等が例示できる。また、G-CSF (granulocyte-macrophage colony-stimulating factor)、TNF-a及びIL-4は、アボトーシス精制剂 (Propranolol) の後フローサイトメーター (EPICS XL: Coulter) にて染色性細胞 (アボトーシス精制剂) を検出し、結果を表12に示す。尚、培地は、RPMI-1640培地 (Life Technologies社製) を使用して用いた。

(00130) 丸剤の形態に成形するに際しては、相体としてこの分野で後半段のものを広く使用でき、例えばブドウ糖、カカオオイル、硬化植物油、カオチアゴム末、アラビアゴム末、セラチン等の結合剤、ミナミカンタン等の増強剤等が例示できる。また、G-CSF (granulocyte-macrophage colony-stimulating factor)、TNF-a及びIL-4は、アボトーシス精制剂 (Propranolol) の後フローサイトメーター (EPICS XL: Coulter) にて染色性細胞 (アボトーシス精制剂) を検出し、結果を表13に示す。尚、培地は、RPMI-1640培地 (Life Technologies社製) を使用して用いた。

特開2002-128690

(5)

6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74  
75  
76  
77  
78  
79  
80  
81  
82  
83  
84  
85  
86  
87  
88  
89  
90  
91  
92  
93  
94  
95  
96  
97  
98  
99  
100  
101  
102  
103  
104  
105  
106  
107  
108  
109  
110  
111  
112  
113  
114  
115  
116  
117  
118  
119  
120  
121  
122  
123  
124  
125  
126  
127  
128  
129  
130  
131  
132  
133  
134  
135  
136  
137  
138  
139  
140  
141  
142  
143  
144  
145  
146  
147  
148  
149  
150  
151  
152  
153  
154  
155  
156  
157  
158  
159  
160  
161  
162  
163  
164  
165  
166  
167  
168  
169  
170  
171  
172  
173  
174  
175  
176  
177  
178  
179  
180  
181  
182  
183  
184  
185  
186  
187  
188  
189  
190  
191  
192  
193  
194  
195  
196  
197  
198  
199  
200  
201  
202  
203  
204  
205  
206  
207  
208  
209  
210  
211  
212  
213  
214  
215  
216  
217  
218  
219  
220  
221  
222  
223  
224  
225  
226  
227  
228  
229  
230  
231  
232  
233  
234  
235  
236  
237  
238  
239  
240  
241  
242  
243  
244  
245  
246  
247  
248  
249  
250  
251  
252  
253  
254  
255  
256  
257  
258  
259  
259  
260  
261  
262  
263  
264  
265  
266  
267  
268  
269  
270  
271  
272  
273  
274  
275  
276  
277  
278  
279  
280  
281  
282  
283  
284  
285  
286  
287  
288  
289  
289  
290  
291  
292  
293  
294  
295  
296  
297  
298  
299  
299  
300  
301  
302  
303  
304  
305  
306  
307  
308  
309  
309  
310  
311  
312  
313  
314  
315  
316  
317  
318  
319  
319  
320  
321  
322  
323  
324  
325  
326  
327  
328  
329  
329  
330  
331  
332  
333  
334  
335  
336  
337  
338  
339  
339  
340  
341  
342  
343  
344  
345  
346  
347  
348  
349  
349  
350  
351  
352  
353  
354  
355  
356  
357  
358  
359  
359  
360  
361  
362  
363  
364  
365  
366  
367  
368  
369  
369  
370  
371  
372  
373  
374  
375  
376  
377  
378  
379  
379  
380  
381  
382  
383  
384  
385  
386  
387  
388  
389  
389  
390  
391  
392  
393  
394  
395  
396  
397  
398  
399  
399  
400  
401  
402  
403  
404  
405  
406  
407  
408  
409  
409  
410  
411  
412  
413  
414  
415  
416  
417  
418  
419  
419  
420  
421  
422  
423  
424  
425  
426  
427  
428  
429  
429  
430  
431  
432  
433  
434  
435  
436  
437  
438  
439  
439  
440  
441  
442  
443  
444  
445  
446  
447  
448  
449  
449  
450  
451  
452  
453  
454  
455  
456  
457  
458  
459  
459  
460  
461  
462  
463  
464  
465  
466  
467  
468  
469  
469  
470  
471  
472  
473  
474  
475  
476  
477  
478  
479  
479  
480  
481  
482  
483  
484  
485  
486  
487  
488  
489  
489  
490  
491  
492  
493  
494  
495  
496  
497  
498  
499  
499  
500  
501  
502  
503  
504  
505  
506  
507  
508  
509  
509  
510  
511  
512  
513  
514  
515  
516  
517  
518  
519  
519  
520  
521  
522  
523  
524  
525  
526  
527  
528  
529  
529  
530  
531  
532  
533  
534  
535  
536  
537  
538  
539  
539  
540  
541  
542  
543  
544  
545  
546  
547  
548  
549  
549  
550  
551  
552  
553  
554  
555  
556  
557  
558  
559  
559  
560  
561  
562  
563  
564  
565  
566  
567  
568  
569  
569  
570  
571  
572  
573  
574  
575  
576  
577  
578  
579  
579  
580  
581  
582  
583  
584  
585  
586  
587  
588  
589  
589  
590  
591  
592  
593  
594  
595  
596  
597  
598  
599  
599  
600  
601  
602  
603  
604  
605  
606  
607  
608  
609  
609  
610  
611  
612  
613  
614  
615  
616  
617  
618  
619  
619  
620  
621  
622  
623  
624  
625  
626  
627  
628  
629  
629  
630  
631  
632  
633  
634  
635  
636  
637  
638  
639  
639  
640  
641  
642  
643  
644  
645  
646  
647  
648  
649  
649  
650  
651  
652  
653  
654  
655  
656  
657  
658  
659  
659  
660  
661  
662  
663  
664  
665  
666  
667  
668  
669  
669  
670  
671  
672  
673  
674  
675  
676  
677  
678  
679  
679  
680  
681  
682  
683  
684  
685  
686  
687  
688  
689  
689  
690  
691  
692  
693  
694  
695  
696  
697  
698  
699  
699  
700  
701  
702  
703  
704  
705  
706  
707  
708  
709  
709  
710  
711  
712  
713  
714  
715  
716  
717  
718  
719  
719  
720  
721  
722  
723  
724  
725  
726  
727  
728  
729  
729  
730  
731  
732  
733  
734  
735  
736  
737  
738  
739  
739  
740  
741  
742  
743  
744  
745  
746  
747  
748  
749  
749  
750  
751  
752  
753  
754  
755  
756  
757  
758  
759  
759  
760  
761  
762  
763  
764  
765  
766  
767  
768  
769  
769  
770  
771  
772  
773  
774  
775  
776  
777  
778  
779  
779  
780  
781  
782  
783  
784  
785  
786  
787  
788  
789  
789  
790  
791  
792  
793  
794  
795  
796  
797  
798  
799  
799  
800  
801  
802  
803  
804  
805  
806  
807  
808  
809  
809  
810  
811  
812  
813  
814  
815  
816  
817  
818  
819  
819  
820  
821  
822  
823  
824  
825  
826  
827  
828  
829  
829  
830  
831  
832  
833  
834  
835  
836  
837  
838  
839  
839  
840  
841  
842  
843  
844  
845  
846  
847  
848  
849  
849  
850  
851  
852  
853  
854  
855  
856  
857  
858  
859  
859  
860  
861  
862  
863  
864  
865  
866  
867  
868  
869  
869  
870  
871  
872  
873  
874  
875  
876  
877  
878  
879  
879  
880  
881  
882  
883  
884  
885  
886  
887  
888  
889  
889  
890  
891  
892  
893  
894  
895  
896  
897  
898  
899  
899  
900  
901  
902  
903  
904  
905  
906  
907  
908  
909  
909  
910  
911  
912  
913  
914  
915  
916  
917  
918  
919  
919  
920  
921  
922  
923  
924  
925  
926  
927  
928  
929  
929  
930  
931  
932  
933  
934  
935  
936  
937  
938  
939  
939  
940  
941  
942  
943  
944  
945  
946  
947  
948  
949  
949  
950  
951  
952  
953  
954  
955  
956  
957  
958  
959  
959  
960  
961  
962  
963  
964  
965  
966  
967  
968  
969  
969  
970  
971  
972  
973  
974  
975  
976  
977  
978  
979  
979  
980  
981  
982  
983  
984  
985  
986  
987  
988  
989  
989  
990  
991  
992  
993  
994  
995  
996  
997  
998  
999  
999  
1000  
1001  
1002  
1003  
1004  
1005  
1006  
1007  
1008  
1009  
1009  
1010  
1011  
1012  
1013  
1014  
1015  
1016  
1017  
1018  
1019  
1019  
1020  
1021  
1022  
1023  
1024  
1025  
1026  
1027  
1028  
1029  
1029  
1030  
1031  
1032  
1033  
1034  
1035  
1036  
1037  
1038  
1039  
1039  
1040  
1041  
1042  
1043  
1044  
1045  
1046  
1047  
1048  
1049  
1049  
1050  
1051  
1052  
1053  
1054  
1055  
1056  
1057  
1058  
1059  
1059  
1060  
1061  
1062  
1063  
1064  
1065  
1066  
1067  
1068  
1069  
1069  
1070  
1071  
1072  
1073  
1074  
1075  
1076  
1077  
1078  
1079  
1079  
1080  
1081  
1082  
1083  
1084  
1085  
1086  
1087  
1088  
1089  
1089  
1090  
1091  
1092  
1093  
1094  
1095  
1096  
1097  
1098  
1098  
1099  
1099  
1100  
1101  
1102  
1103  
1104  
1105  
1106  
1107  
1108  
1109  
1109  
1110  
1111  
1112  
1113  
1114  
1115  
1116  
1117  
1118  
1119  
1119  
1120  
1121  
1122  
1123  
1124  
1125  
1126  
1127  
1128  
1129  
1129  
1130  
1131  
1132  
1133  
1134  
1135  
1136  
1137  
1138  
1139  
1139  
1140  
1141  
1142  
1143  
1144  
1145  
1146  
1147  
1148  
1149  
1149  
1150  
1151  
1152  
1153  
1154  
1155  
1156  
1157  
1158  
1159  
1159  
1160  
1161  
1162  
1163  
1164  
1165  
1166  
1167  
1168  
1169  
1169  
1170  
1171  
1172  
1173  
1174  
1175  
1176  
1177  
1178  
1179  
1179  
1180  
1181  
1182  
1183  
1184  
1185  
1186  
1187  
1188  
1189  
1189  
1190  
1191  
1192  
1193  
1194  
1195  
1196  
1197  
1198  
1198  
1199  
1199  
1200  
1201  
1202  
1203  
1204  
1205  
1206  
1207  
1208  
1209  
1209  
1210  
1211  
1212  
1213  
1214  
1215  
1216  
1217  
1218  
1219  
1219  
1220  
1221  
1222  
1223  
1224  
1225  
1226  
1227  
1228  
1229  
1229  
1230  
1231  
1232  
1233  
1234  
1235  
1236  
1237  
1238  
1239  
1239  
1240  
1241  
1242  
1243  
1244  
1245  
1246  
1247  
1248  
1249  
1249  
1250  
1251  
1252  
1253  
1254  
1255  
1256  
1257  
1258  
1259  
1259  
1260  
1261  
1262  
1263  
1264  
1265  
1266  
1267  
1268  
1269  
1269  
1270  
1271  
1272  
1273  
1274  
1275  
1276  
1277  
1278  
1279  
1279  
1280  
1281  
1282  
1283  
1284  
1285  
1286  
1287  
1288  
1289  
1289  
1290  
1291  
1292  
1293  
1294  
1295  
1296  
1297  
1298  
1298  
1299  
1299  
1300  
1301  
1302  
1303  
1304  
1305  
1306  
1307  
1308  
1309  
1309  
1310  
1311  
1312  
1313  
1314  
1315  
1316  
1317  
1318  
1319  
1319  
1320  
1321  
1322  
1323  
1324  
1325  
1326  
1327  
1328  
1329  
1329  
1330  
1331  
1332  
1333  
1334  
1335  
1336  
1337  
1338  
1339  
1339  
1340  
1341  
1342  
1343  
1344  
1345  
1346  
1347  
1348  
1349  
1349  
1350  
1351  
1352  
1353  
1354  
1355  
1356  
1357  
1358  
1359  
1359  
1360  
1361  
1362  
1363  
1364  
1365  
1366  
1367  
1368  
1369  
1369  
1370  
1371  
1372  
1373  
1374  
1375  
1376  
1377  
1378  
1379  
1379  
1380  
1381  
1382  
1383  
1384  
1385  
1386  
1387  
1388  
1389  
1389  
1390  
1391  
1392  
1393  
1394  
1395  
1396  
1397  
1398  
1398  
1399  
1399  
1400  
1401  
1402  
1403  
1404  
1405  
1406  
1407  
1408  
1409  
1409  
1410  
1411  
1412  
1413  
1414  
1415  
1416  
1417  
1418  
1419  
1419  
1420  
1421  
1422  
1423  
1424  
1425  
1426  
1427  
1428  
1429  
1429  
1430  
1431  
1432  
1433  
1434  
1435  
1436  
1437  
1438  
1439  
1439  
1440  
1441  
1442  
1443  
1444  
1445  
1446  
1447  
1448  
1449  
1449  
1450  
1451  
1452  
1453  
1454  
1455  
1456  
1457  
1458  
1459  
1459  
1460  
1461  
1462  
1463  
1464  
1465  
1466  
1467  
1468  
1469  
1469  
1470  
1471  
1472  
1473  
1474  
1475  
1476  
1477  
1478  
1479  
1479  
1480  
1481  
1482  
1483  
1484  
1485  
1486  
1487  
1488  
1489  
1489  
1490  
1491  
1492  
1493  
1494  
1495  
1496  
1497  
1498  
1498  
1499  
1499  
1500  
1501  
1502  
1503  
1504  
1505  
1506  
1507  
1508  
1509  
1509  
1510  
1511  
1512  
1513  
1514  
1515  
1516  
1517  
1518  
1519  
1519  
1520  
1521  
1522  
1523  
1524  
1525  
1526  
1527  
1528  
1529  
1529  
1530  
1531  
1532  
1533  
1534  
1535  
1536  
1537  
1538  
1539  
1539  
1540  
1541  
1542  
1543  
1544  
1545  
1546  
1547  
1548  
1549  
1549  
1550  
1551  
1552  
1553  
1554  
1555  
1556  
1557  
1558  
1559  
1559  
1560  
1561  
1562  
1563  
1564  
1565  
1566  
1567  
1568  
1569  
1569  
1570  
1571  
1572  
1573  
1574  
1575  
1576  
1577  
1578  
1579  
1579  
1580  
1581  
1582  
1583  
1584  
1585  
1586  
1587  
1588  
1589  
1589  
1590  
1591  
1592  
1593  
1594  
1595  
1596  
1597  
1598  
1598  
1599  
1599  
1600  
1601  
1602  
1603  
1604  
1605  
1606  
1607  
1608  
1609  
1609  
1610  
1611  
1612  
1613  
1614  
1615  
1616  
1617  
1618  
1619  
1619  
1620  
1621  
1622  
1623  
1624  
1625  
1626  
1627  
1628  
1629  
1629  
1630  
1631  
1632  
1633  
1634  
1635  
1636  
1637  
1638  
1639  
1639  
1640  
1641  
1642  
1643  
1644  
1645  
1646  
1647  
1648  
1649  
1649  
1650  
1651  
1652  
1653  
1654  
1655  
1656  
1657  
1658  
1659  
1659  
1660  
1661  
1662  
1663  
1664  
1665  
1666  
1667  
1668  
1669  
1669  
1670  
1671  
1672  
1673  
1674  
1675  
1676  
1677  
1678  
1679  
1679  
1680  
1681  
1682  
1683  
1684  
1685  
1686  
1687  
1688  
1689  
1689  
1690  
1691  
1692  
1693  
1694  
1695  
1696  
1697  
1698  
1698  
1699  
1699  
1700  
1701  
1702  
1703  
1704  
1705  
1706  
1707  
1708  
1709  
1709  
1710  
1711  
1712  
1713  
1714  
1715  
1716  
1717  
1718  
1719  
1719  
1720  
1721  
1722  
1723  
1724  
1725  
1726  
1727  
1728  
1729  
1729  
1730  
1731  
1732  
1733  
1734  
1735  
1736  
1737  
1738  
1739  
1739  
1740  
1741  
1742  
1743  
1744  
1745  
1746  
1747  
1748  
1749  
1749  
1750  
1751  
1752  
1753  
1754  
1755  
1756  
1757  
1758  
1759  
1759  
1760  
1761  
1762  
1763  
1764  
1765  
1766  
1767  
1768  
1769  
1769  
1770  
1771  
1772  
1773  
1774  
1775  
1776  
1777  
1778  
1779  
1779  
1780  
1781  
1782  
1783  
1784  
1785  
1786  
1787  
1788  
1789  
1789  
1790  
1791  
1792  
1793  
1794  
1795  
1796  
1797  
1798  
1798  
1799  
1799  
1800  
1801  
1802  
1803  
1804  
1805  
1806  
1807  
1808  
1809  
1809  
1810  
1811  
1812  
1813  
1814  
1815  
1816  
1817  
1818  
1819  
1819  
1820  
1821  
1822  
1823  
1824  
1825  
1826  
1827  
1828  
1829  
1829  
1830  
1831  
1832  
1833  
1834  
1835  
1836  
1837  
1838  
1839  
1839  
1840  
1841  
1842  
1843  
1844  
1845  
1846  
1847  
1848  
1849  
1849  
1850  
1851  
1852  
1853  
1854  
1855  
1856  
1857  
1858  
1859  
1859  
1860  
1861  
1862  
1863  
1864  
1865  
1866  
1867  
1868  
1869  
1869  
1870  
1871  
1872  
1873  
1874  
1875  
1876  
1877  
1878  
1879  
1879  
1880  
1881  
1882  
1883  
1884  
1885  
1886  
1887  
1888  
1889  
1889  
1890  
1891  
1892  
1893  
1894  
1895  
1896  
1897  
1898  
1898  
1899  
1899  
1900  
1901  
1902  
1903  
1904  
1905  
1906  
1907  
1908<br

## PATENT ABSTRACTS OF JAPAN

(11)Publication number : 2002-128690

(43)Date of publication of application : 09.05.2002

(51)Int.Cl.

A61K 38/00  
A61P 29/00  
A61P 35/00  
A61P 43/00

(21)Application number : 2000-316464

(71)Applicant : HIROHATA TOSHINARI

(22)Date of filing : 17.10.2000

(72)Inventor : HIROHATA TOSHINARI

### (54) APOPTOSIS-INDUCING AGENT

(57)Abstract:

PROBLEM TO BE SOLVED: To obtain an apoptosis-inducing agent consisting mainly of TNF- $\alpha$  and IL-4 as active ingredients.

SOLUTION: This apoptosis-inducing agent has a synergistically enhanced apoptosis-inducing effect as compared with the case of using the TNF- $\alpha$  or IL-4 singly and can be used as an anticancer agent, a chronic rheumatoid arthritis-treating agent, an autoimmune disease-treating agent and a treating agent for hepatic diseases such as hepatitis, hepatic cirrhosis, and exhibiting less adverse effects.

### LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

Copyright (C): 1998.2003 Japan Patent Office

\* NOTICES \*

JPO and NCIPI are not responsible for any damages caused by the use of this translation.

- This document has been translated by computer. So the translation may not reflect the original precisely.  
2.\*\*\* shows the word which can not be translated.  
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]

[Claim 1] The apoptosis inducer which makes TNF-alpha and IL-4 an active principle.

[Claim 2] The apoptosis inducer according to claim 1 which is a malignant tumor; or prevention and the remedy of rheumatoid arthritis.

[Translation done.]

## \* NOTICES \*

JPO and WIPO are not responsible for any damages caused by the use of this translation.

1. This document has been translated by computer. So the translation may not reflect the original precisely.
2. \*\*\* shows the word which can not be translated.
3. In the drawings, any words are not translated.

**DETAILED DESCRIPTION****[Detailed Description of the Invention]****[Field of the Invention]** This invention relates to an apoptosis inducer.**[0001]**

**[Description of the Prior Art]** Apoptosis is one gestalt of the programmed cell death, and is contrasted with classic cell death (necrosis). Apoptosis happens to the bottom of the condition of the versatility on physiology. As the morphological description Condensation and pyknosis of chromatin relevant to lack of contact into a surrounding cell, the inspissation of cytoplasm, and the activity of endonuclease. Nuclear segmentation, disappearance of the microvillus of cell surface, smoothing (blistering of cell surface: membrane blebbing) of cell surface, and fragmentation of DNA by ENDONUClease are observed. It is discussed as a device in which the engulfment is carried out by the cell which the last fragment of an APOTIKKU somatic cell adions [Duvall, E. and Wyllie, A.H., Immunology Today, 7 (4) --> 115-119(1986); Science, 245:301--305 (1989)].

[0003] Although apoptosis is physiological cell death indispensable to normal generating and differentiation and it has happened to each cell in the cell kinetic of a normal body tissue etc., in diseases, such as a malignant tumor, leukemia, a growth sex skin skin disease, rheumatoid arthritis, and an autoimmune disease, apoptosis is controlled superfluously, consequently it is thought that a functional disorder arises into a cell. For example, WATANABE-FUKUNAGA and others is in the Fas molecule which participates in apoptosis about abnormalities in a MR10pr/[pr mouse, and the negative selection (apoptosis) device of the self-reactivity T cell in a thymus gland did not operate well, but it is suggested that the symptoms of an autoimmune disease develop as a result (Watanabe-Fukunaga, R. et al., Nature, 356:314-317 (1992), moreover — the process in which the chronic hepatitis shifts to liver cirrhosis and hepatic carcinoma — apoptosis — a control condition — it is — this — a site — an ibis — it is thought that it progresses to the fibrosis and the liver cirrhosis following the inflammation of the hepatocyte by the chic T cell. Therefore, the matter which guides the apoptosis of a cell which participates in this disease is useful as prevention and a remedy of the disease concerned.

[0004] The actinomycin-D which is the cycloheximide and inhibitor of RNA synthesis which are the former and a protein synthesis inhibitor, it reports that cytokine, such as a tumor necrosis factor (henceforth "TNF-alpha") and lymphotoxin (LT), has an induction operation of apoptosis — having (Marin, S.J., et al., J.Immunol., 145, and 1859-1867 (1990) —) Strelow, A., et al., J.Exp.Med, 192, 601-611 (2000). Recently, moreover, to interleukin 4 (henceforth "IL-4") it is reported that there is an apoptosis induction operation to Homo sapiens monocyte or eosinophile leucocyte (J. — Immuno, and 148 (6) —) 1812-1816 (1992). J.Allergy Clin.Immunol., 102 (6 Pt 1), 1013-1020 (1998).

[0005] However, the matter known until now was not enough from the point of apoptosis induction activity or a side effect, and the apoptosis inducer with high safety with high and apoptosis induction activity was called for.

[0006] **[Problem(s) to be Solved by the Invention]** This invention aims to let effectiveness offer a high

apoptosis inducer with high safety.

**[0007]**

**[Means for Solving the Problem]** The apoptosis inductive effect which each has to an undifferentiated cell or a precursor cell was reinforced in multiplication, and this inventions completed a header and this invention for it being useful as prevention and a remedy of the disease accompanying superfluous control of apoptosis, when IL-4 were used together with TNF-alpha, as a result of inquiring wholeheartedly in view of this actual condition about the matter which has apoptosis induction activity.

[0008] That is, this invention offers the apoptosis inducer which makes TNF-alpha and IL-4 an active principle.

**[0009]**

**[Embodiment of the Invention]** Although the apoptosis inducer of this invention makes TNF-alpha and IL-4 an active principle With TNF-alpha, focusing on the living body defense mechanism through inflammation here Antitumor action. An osteoclasia operation, the incorporation inhibitory action of the lipid to a cell, a production induction operation of interleukin 1 and a colony stimulating factor, etc., It is the polypeptide of molecular-weight 17kDa which shows various biobioactive, and is a kind of the cytokine which has antitumor action, an I-beam allergy induction operation, anti-inflammatory activity, etc., focusing on the immunocyte stimulation (differentiation to the plasma cell of a B cell, differentiation growth of a T cell) of the large range in IL-4. As mentioned above, it is reported to these TNF-alpha and IL-4 that there is an apoptosis induction operation, but when IL-4 are used together with TNF-alpha, this apoptosis inductive effect's being reinforced in multiplication is that completely predicting became impossible.

[0010] any of the recombinant produced by the natural mold or gene recombination which has the activity as TNF-alpha and IL-4, respectively as TNF-alpha used for the apoptosis inducer of this invention, and IL-4 — although — it is included.

[0011] TNF-alpha of a natural mold can be obtained by refining according to the known approaches, such as affinity chromatography and HPLC, from the culture supernatant of the existing cell strains, such as Sendai Virus (Sendai Virus) stimulus Homo sapiens B lymphoblast stock B ALL-1, and TNF-alpha obtained by recombination of a gene can be obtained by refining similarly the Escherichia coli and the production protein of the existing cell strain which introduced the plasmid or vector incorporating a known gene.

[0012] Moreover, by mitogen etc., a Homo sapiens T cell clone, or the existing cell strain of arbitration is refined similarly, and can acquire from un-stimulating or the stimulated culture supernatant, and natural IL-4 can obtain recombination IL-4 as well as the above by use of the existing gene.

[0013] The apoptosis inducer of this invention may be prepared so that TNF-alpha and IL-4 may be contained in single pharmaceutical preparation, or it may prepare TNF-alpha and each of IL-4 as separate pharmaceutical preparation, and may use these two pharmaceutical preparation together. Moreover, as for TNF-alpha and the rate of a compounding ratio of IL-4, it is desirable not to be limited especially if the synergistic effect of an apoptosis induction operation can be demonstrated, but to especially be mixed in 1 - 99% of range, respectively, to blend TNF-alpha and to blend IL-4 at 70 - 30% 30 to 70%.

[0014] Thus, the prepared apoptosis inducer of this invention demonstrates the synergism that apotosis inductive effect is reinforced remarkably, compared with the case where TNF-alpha or IL-4 are independently used as shown in the after-mentioned example. Therefore, compared with the case where TNF-alpha or IL-4 are independently prescribed for the patient, both dose can be decreased sharply, and it becomes mitigable [ a side effect ].

[0015] Although the dose per [ to the adult of the apoptosis inducer of this invention ] day is chosen suitably broadly, it is usually 50microg/body per day - 50 mg/body extent about TNF-alpha, and it is desirable about IL-4 to consider as 50microg/body per day - 50 mg/body extent.

[0016] The apoptosis inducer of this invention is used according to that purpose of use with various kinds of administration Gestalten commonly used in this field as physic pharmaceutical preparation. This pharmaceutical preparation is prepared using a diluent or excipients, such as

the bulking agent usually used, an extending agent, a binder, a \*\*\*\* agent, disintegrator, a surface active agent, and lubricant, as avirulent pharmacology support. Dosage forms can choose various kinds of gestalten according to the therapy purpose, and a tablet, a pill, powder, liquids and solutions, suspension, an emulsion, a granule, a capsule, suppositories, injections (liquids and solutions, suspension, etc.), ophthalmic solutions, etc. are mentioned as this typical thing.

[0017] It faces fabricating in the gestalt of a tablet and a well-known thing can be conveniently used widely in this field as support. For example, a lactose, white soft sugar, a sodium chloride, grape sugar, a urea, starch, a calcium carbonate, Excipients, such as a kaolin, crystalline cellulose, and a silicic acid, water, ethanol, propanol, Simple syrup, grape-sugar liquid, starch liquid, a gelatin solution, a carboxymethyl cellulose, A shellac, methyl cellulose, potassium phosphate, the binder of polyvinyl-pyrrolidone sugar, Desiccation starch, sodium alginate, agar powder, the end of a laminarin, A sodium hydrogencarbonate, a calcium carbonate, and polyoxyethylene sorbitan fatty acid ester Sodium lauryl sulfate, a stearin a acid monoglyceride, starch, Collapse inhibitors, such as disintegrator, such as a lactose, white soft sugar, stearin, cocoa butter, and hydrogenated oil. Absorption enhancers, such as a quaternary-ammonium-salt radical and sodium lauryl sulfate. Lubricant, such as a polyethylene glycol, etc. can be illustrated in absorbents, such as moisturizers, such as a glycerol and starch, starch, a lactose, a kaolin, a bentonite, and a colloid silicic acid, purification talc, a stearate, and the end of a boric acid. Furthermore, a tablet can be used as the tablet which gave the usual coating if needed, for example, a sugar-coated tablet, a gelatin encapsulation lock, an enteric tablet, a film coated tablet or an auxiliary rim lock, and a multilayered tablet.

[0018] It can face fabricating in the gestalt of a pill, and a thing conventionally well-known in this field as support can be used widely, for example, disintegrator, such as binders, such as excipients, such as grape sugar, a lactose, starch, cacao butter, hardening vegetable oil, a kaolin, and talc, gummi arabicum pulvratum, powdered tragacanth, gelatin, and ethanol, and laminaran agar, etc. can be illustrated.

[0019] It can face fabricating in the gestalt of suppositories, and a conventionally well-known thing can be widely used as support, for example, the ester of a polyethylene glycol, cacao butter, higher alcohol, and higher alcohol, gelatin, semisynthetic glyceride, etc. can be mentioned.

[0020] When prepared as injections, liquids and solutions and suspension are sterilized, and it is desirable that they are blood and an isotonicity, and they can be faced fabricating in the gestalt of these liquids and solutions, an emulsion, and suspension, and can use all the things commonly used in this field as diluent, for example, can mention water, ethyl alcohol, propylene glycol, ethoxylated isostearyl alcohol, polyglycidyl isostearyl alcohol, and polyoxethylen sorbitan fatty acid ester. In addition, the salt, the grape sugar, or the glycerol of sufficient amount to prepare an isosmotic solution in this case may be made to contain in physic pharmaceutical preparation, and the usual solubilizing agent, a buffer, an aperient agent, etc. may be added.

[0021] Furthermore, a coloring agent, a preservative, perfume, a flavor agent, a sweetening agent, etc. and other drugs may be made to contain if needed in this invention apoptosis inducer.

[0022] The apoptosis inducer of this invention obtained in this way can be applied to the various diseases resulting from superfluous control of apoptosis based on an apoptosis induction operation, and can expect the desired pharmacology effectiveness. As this application disease, for example, Cancer, AIDS, ARC (AIDS associated diseases), ATL (adult T-cell leukemia), Adult T-lymphocytoma, Hair Mr. cellularia leukemia (Hairy cell leukemia), the myelosis (HAM/TSP), HTLV-1 associated diseases, such as respiratory disorder (HAB/HABA), arthrosis (HAAP), and uveitis (HAU), Collagen diseases, such as an autoimmune disease (systemic lupus erythematosus), for example, SLE, and rheumatoid arthritis (RA). Ulcerative colitis, Sjogren's syndrome, primary biliary liver cirrhosis, an outbreak thrombocytopenic purpura (Idiopathic Thrombocytopenic Purpura(ITP)), Autoimmune hemolytic anemia, Hashimoto's disease, insulin-dependent (I-beam) diabetes mellitus, etc. can be illustrated. The apoptosis inducer of this invention Moreover, myelodysplastic syndromes, periodicity thrombocytopenia. Various kinds of diseases accompanied by thrombocytopenia, such as aplastic anemia, outbreak thrombocytopenia, and disseminated intravascular coagulation, Various kinds of hepatitis, such

as C mold, A mold, B mold, and a female mold, an Alzheimer disease, the Alzheimer mold senility Alzheimer's disease, Myocarditis, ARDS (adult respiratory urgency syndrome), an infectious disease, liver cirrhosis, prostatomegaly, It can be adapted also for various diseases, such as fibroid, bronchial asthma, arteriosclerosis, various congenital malformation, a nephritis, senile cataract, chronic fatigue syndrome (Chronic Fatigued Syndrome), and myotrophia dystonia (Myotrophic dystrophy).

[0023] When using this invention apoptosis inducer as an anticancer agent especially, apoptosis can be guided to a cancer cell by the administration, a carcinostatic operation is demonstrated, but if this is used together with various kinds of anticancer agents and radiotherapy which are known as a chemotherapeutic drug of cancer, the carcinostatic effectiveness can be promoted further and mitigation of a side effect can also be aimed at. As this chemotherapeutic drug, 5-fluorouracil (5-FU, consonance fermentation industrial incorporated company make), a mitomycin (Mitomycin-C, shrine make same as the above), futuraf (FT-207, Taiho Pharmaceutical, Inc. make), endoxan (Endoxan, Shionogi & Co., Ltd. make), a tomyomycin (Tozymycin, Takeda Chemical Industries, Ltd. make), etc. are mentioned, for example.

[0024]

[Example] An example is given to below and this invention is further explained to a detail. The monocyte was separated from an example 1(1) activity mold rheumatoid arthritis patient's bone marrow specimen by the concentration gradient method using the commercial kit (product made from Histopaque-Sigma). CD34 positivity cell (CD34+) was obtained from the separated monocyte using the magnetic bead (product made from Dynal CD34 progenitor cells selection system Dynal). As for the cell which carried out separation recovery, the CD34+ cell of the CD19+ B cell was 0.5% or less at about 95%.

[0025] (2) Prepare by the culture medium which added SCF (10 ng/ml) and GM-CSF (1 ng/ml) so that it might become 1.0x10<sup>5</sup> / well to a flat bottom microplate (No.3596:Costar) 24 well about a CD34+ cell. TNF-alpha (10 ng/ml) addition, IL-4 (10 ng/ml) addition, and its both (10 ng/ml+10ng/ml) were cultivated for two weeks by addition or un-adding (control group). The cell was washed in PBS after culture, the cell was floated to PBS2000microl containing 0.1% Triton-X-100 and 0.1% sodium citrate, and the dyeing positive cell (apoptosis dead cell) was measured with PI dyeing (10microl of 10microg/ml Propidium Iodide is added, and it is 10 minutes at 4 degrees C) back flow cytometer (EPICS XL:Coulter). A result is shown in Table 1. In addition, the culture medium added and used penicillin G (100 unit/ml) streptomycin (100microg/ml), L-glutamine (0.3mg/ml), and FBS (10% product made from fetalbovine serum: Life Technologies) for RPMI-1640 culture medium (product made from Life Technologies). Moreover, the commercial item (product made from Puro Tech EC) was used for each of SCF (stem cell factor), GM-CSF (granulocyte-macrophage colonystimulating factor), TNF-alpha, and IL-4.

[0026]

[Table 1]

試験群	死細胞率 (%)
対照	2.33
TNF-α	2.08
IL-4	4.03
TNF-α / IL-4	10.7

[0027] From Table 1, it was shown that the rate of a dead cell reinforces in multiplication compared with the case where TNF-alpha or IL-4 are independently used for the apoptosis inducer of this invention which used TNF-alpha and IL-4 together.

[0028] TNF-alpha and the combined effect of IL-4 as well as an example 1 (2) were examined using the example 2(Hela cell (uterine cervix squamous-cell-carcinoma origin Homo sapiens cultured cell). In addition, the Hela cell was cultivated on the plate 96 well in 2x10<sup>4</sup>/a well. A result is shown in Table 2.

[Table 2]

		死細胞率 (P1陽性細胞) (%)		
		2日目	3日目	4日目
試験群		TGF-β/IL-6/IFN-γ	TGF-β/IL-6/CSF	TGF-β/IL-6/CSF + IL-10
対照		7.3	11.5	6.9
TNF-α		10.2	15.0	9.4
IL-4		10.1	10.3	8.3
TNF-α/IL-4		18.1	23.1	14.3
				18.8

[0030] From Table 2, it was shown that the rate of a dead cell reinforces in multiplication compared with the case where TNF-alpha or IL-4 are independently used for the apoptosis inducer of this invention which used TNF-alpha and IL-4 together.

[0031] [Effect of the Invention] Compared with the case where TNF-alpha or IL-4 are used independently, apoptosis inductive effect is reinforced in multiplication and can use this invention apoptosis inducer as liver disease therapy agents, such as an anticancer agent with few side effects, a rheumatoid arthritis therapy agent, an autoimmune disease therapy agent, hepatitis, and liver cirrhosis, etc.

[Translation done.]